Looking for a Clean Energy Source? Try Photosynthesis.

Want Oxygen? Try Photosynthesis.

Reaction Center X-ray Crystallography

Annemarie B. Wöhri, Gergely Katona, Linda C. Johansson, Emelie Fritz, Erik Malmerberg, Magnus Andersson, Jonathan Vincent, Mattias Eklund, Marco Cammarata, Michael Wulff, Jan Davidsson, Gerrit Groenhof, Richard Neutze (2010)Light-induced structural changes in a photosynthetic reaction center caught by Laue diffraction. Science 328 (5978):630–633.

Photosynthesis
SC/BIOL 4160

Want to Eat? Try Photosynthesis.

Want to Remove Excess Carbon Dioxide? Try Photosynthesis.

REACTION CENTERS

we have explored the properties of light and the photosynthetic pigments which absorb light

In addition to absorption:

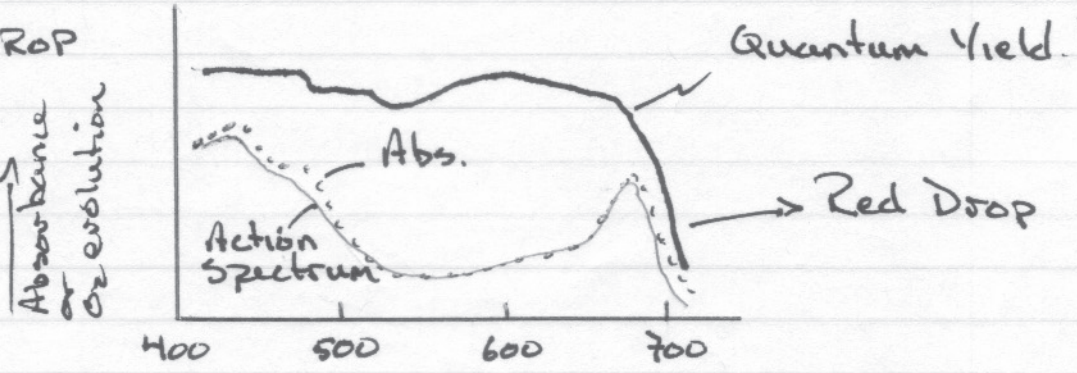
$$h\nu + Chl \longrightarrow Chl^*$$

and transfer of exciton energy from one pigment molecule to another, there is another fate of the exciton: photochemistry

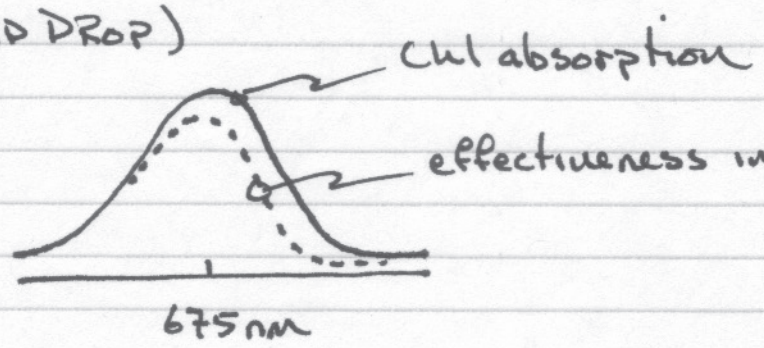
$$Chl \xrightarrow{h\nu} Chl^* \longrightarrow Chl^+ + e^-$$

Photochemistry occurs in the reaction centers.

In higher plants and algae, there are two reaction centers. The evidence for this arose from the observation of the "RED DROP" & RED LIGHT ENHANCEMENT. (Blankenship : page 36-40)



(RED DROP)



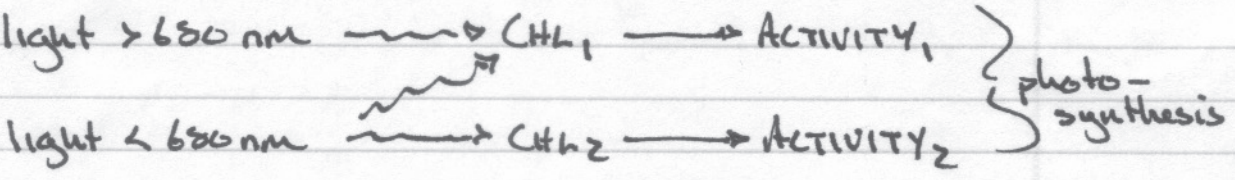
RED LIGHT ENHANCEMENT

< 680 nm light yields a certain level of activity X
> 680 nm " " . . " . . Y

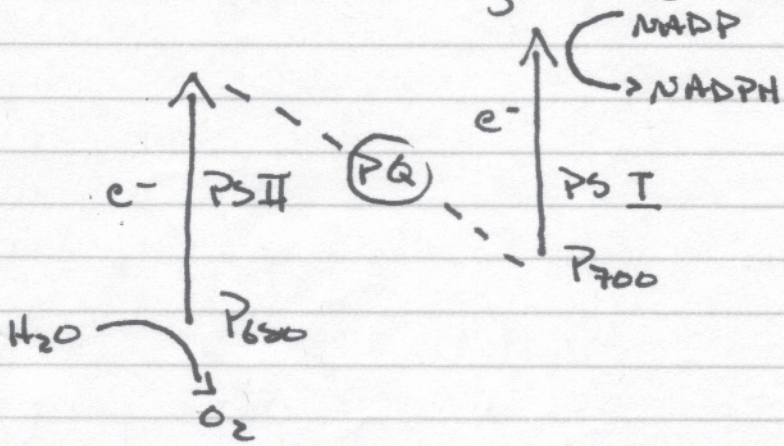
< 680 nm PLUS > 680 nm yields more activity than
X + Y

Thus, red light (> 680 nm) enhancement.

This can be explained by two separate photosystems



This is now formalized by the 'Z' scheme of photosynthesis



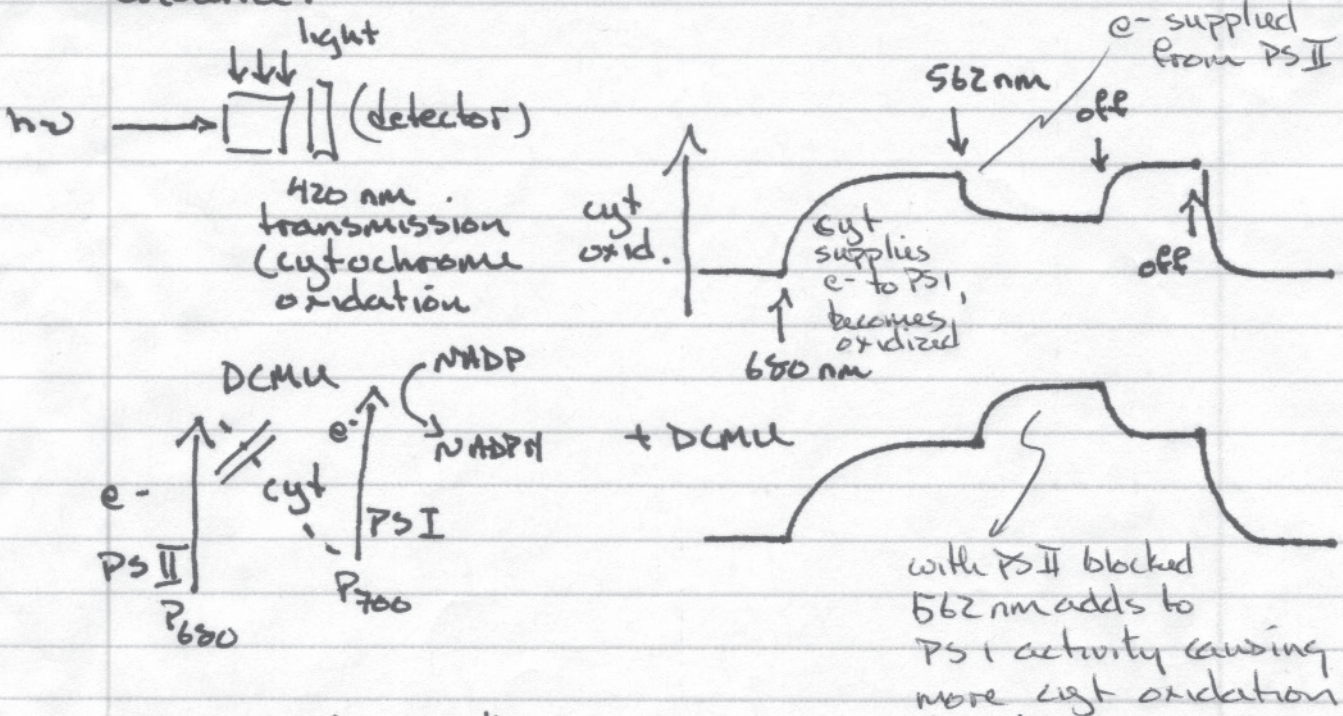
Additional support for two reaction centers (photosystems) comes from absorption spectra Specifically, difference spectra (ΔA) under conditions in which 1) most chlorophyll has been removed with mild detergent, and 2) ΔA in response to light flashes (the ΔA will occur as a consequence of photobleaching ④) in which the ΔA (very small) is averaged over many light flashes.

④ $chl + A \xrightarrow{light} chl^+ + A^-$

photobleached (e⁻ is not available for absorption).

charge separation.

Finally, perturbing the photosystems with inhibitors, and monitoring redox intermediates is supporting evidence.



What are the reaction centers. some structures have been solved

P_{680} and P_{700} (PS I and PS II) Photosynthesis[1]

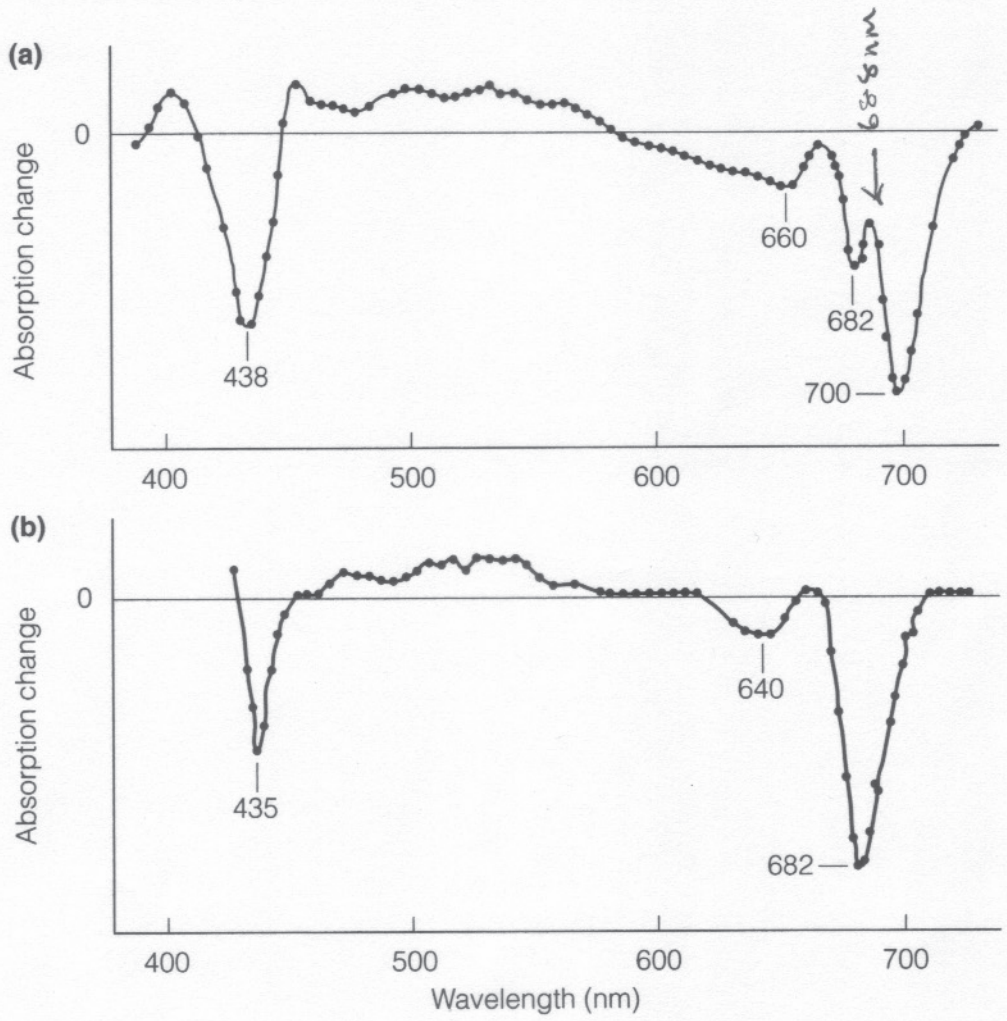


Figure 3.4. Difference spectrum of spinach chloroplasts between light and dark: (a) the change at 700 nm is due to absorption by PSI reaction center chl *a* (after Kok, 1961). (b) The reaction center chl *a* absorption of PSII (P680) (after Döring *et al.*, 1969)

In isolated spinach chloroplasts from which most of the chlorophyll had been removed, difference spectra showed a decrease in absorption (called photobleaching) near 700 nm following illumination (*Figure 3.4*). The signal was altered by the redox state of the chlorophyll; when oxidized there was a loss of absorption at 680 and 690 nm and an increase at 686. The form of chl *a* responsible is called P700 (P for pigment and the wavelength of the absorption change) and is the RC for photosystem I (PSI) (Section 5.3). Only one in 300–400 chlorophyll molecules is in the special form, P700. Difference spectra also show fast change (60 ps) in absorption at 680 nm, separate from, but equivalent to the P700; it is associated with the RC chl *a* of photosystem II (PSII) and is called P680.

[1] Source: DW Lawlor (2001) Photosynthesis. 3[d] edition. Springer-Verlag. pp. 38–39.

REACTION CENTER STRUCTURES.

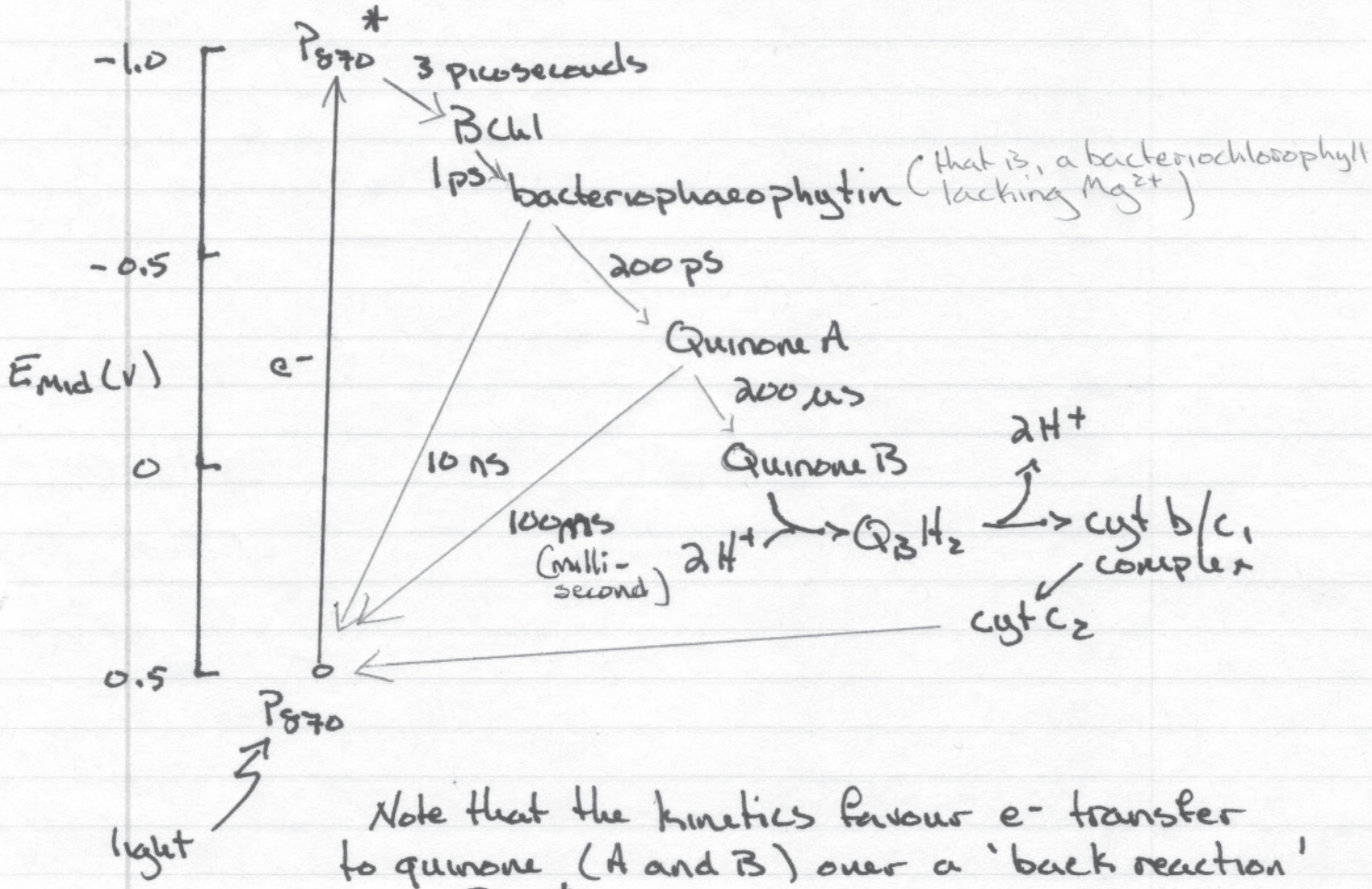
Unlike the two reaction centers of higher plants and algae, the anoxygenic purple bacteria have only one.

This was crucial in the initial elucidation of photochemistry, including direct evidence for the existence of reaction centers.

Rod Clayton (reprint on web site) was one of the first to realize it was possible to isolate reaction centers from purple bacteria (Rhodobacter sphaeroides).

Not only did this allow spectroscopic measurements of the kinetic events in the reaction center but it also led, eventually, to crystallization and x-ray crystallographic solution of the structure of the reaction center, and finally, a Nobel Prize.
(Deisenhofer and Michel 1989 The photosynthetic reaction center from the purple bacterium Rhodopseudomonas viridis. Science 245:1463-1473

Energy-kinetics of the reaction center:



Note that the kinetics favour e⁻ transfer to quinone (A and B) over a 'back reaction' to P_{870}^+

Electrons are normally "returned" to P_{870}^+ from cyt c_2. In Rhodopseudomonas, the net result is the generation of a H^+ gradient (proton motive force) across the membrane. This pmf is used to synthesize ATP (the ATP synthetase).

Deisenhofer J. and H. Michel (1989) The photosynthetic reaction center from the purple bacterium *Rhodopseudomonas viridis*. Science 245:1463-1473. [Nobel Prize Lecture]

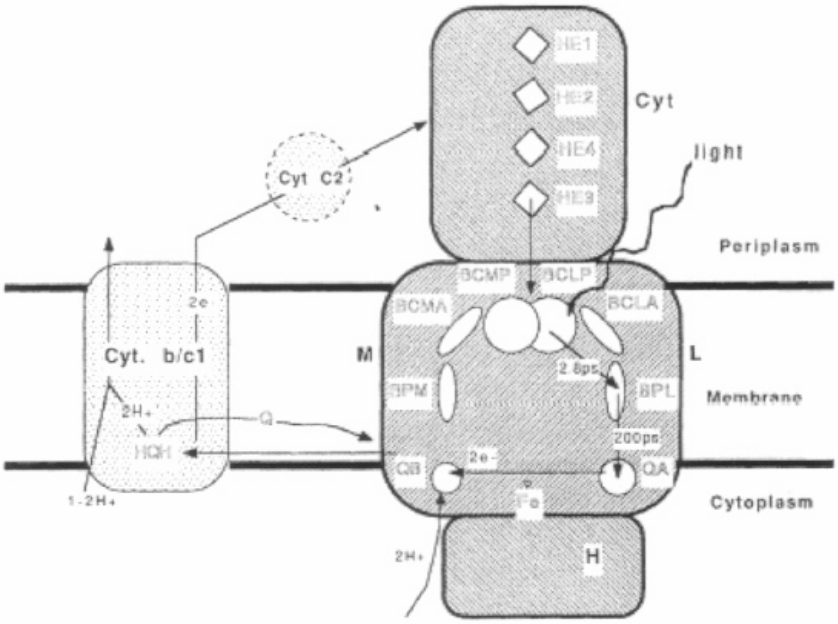


Fig. 6. Schematic view of the reaction center showing the light-driven cyclic electron flow.

The positioning of the components involved directly in light excitation transfer and electron transfer are shown: both relative position and orientation, as well as the kinetics of energy transfer. Orientation is important for the efficiency of energy transfer. The energy transfers are extremely rapid, on the order of 10^{-12} seconds (pico seconds).

DIGRESSION: REDOX REACTIONS.

As we venture into photochemistry, energy transfers no longer occur as dipole-dipole interactions due to absorption/fluorescence of photons (excitons) but as electron transfers between donors and acceptors:

$$Chl \xrightarrow{h\nu} Chl^* \rightleftharpoons Chl^+ + A^-$$

(donor) (acceptor)

Hence, a redox (reduction/oxidation) reaction.

Generically:

$$A + e^- \rightleftharpoons A^-$$

the signs (charge) can vary:

$$A^+ + e^- \rightleftharpoons A$$

as can the stoichiometry:

$$A^+ + 2e^- \rightleftharpoons A^-$$

and, H^+ may be involved:

$$H^+ + A + e^- \rightleftharpoons A{\cdot}H$$

{ in which case the reaction will be pH dependent }

In all cases: $ox + e^- \rightleftharpoons red$ is the fundamental process

Digression: Redox Reactions

For the generic reaction (species j)

$$\text{oxidized}_j + e^- \rightleftharpoons \text{reduced}_j$$

The Redox potential is: (the midpoint potential)

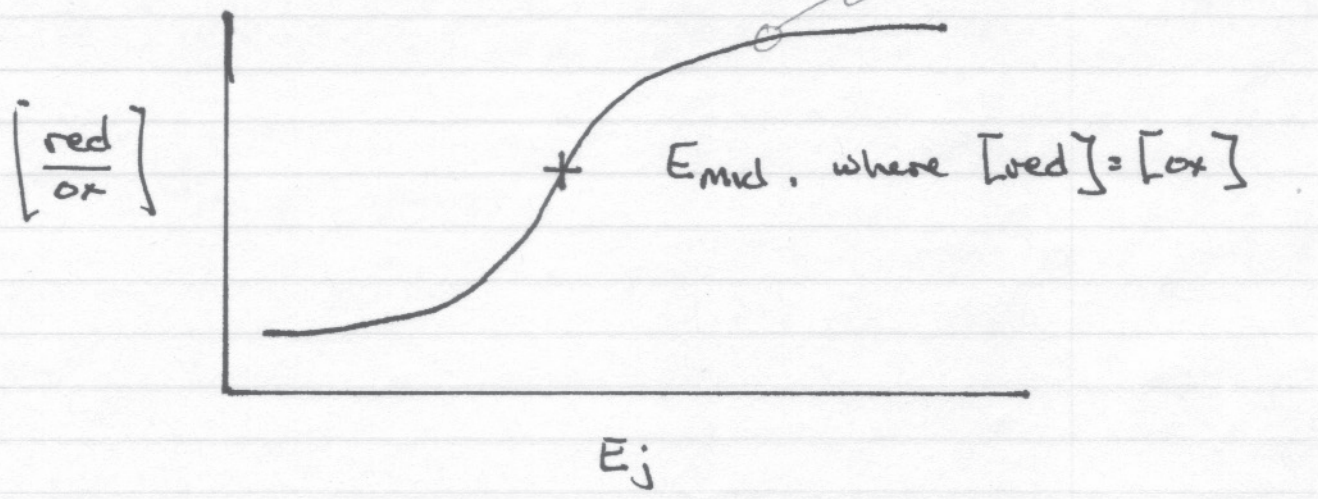
$$E_j = E^j_{mid} - \frac{RT}{qF} \ln\left[\frac{[\text{reduced}_j]}{[\text{oxidized}_j]}\right]$$

(q = number of e-)

$\frac{RT}{F} = 25$ mV at 20°C

Nota bene: ln is the natural logarithm. If $\log_{10}$ is used, $RT/F \simeq 58$ mV.

This relation can be graphed: $\frac{red}{ox} = \exp\left(\frac{qF(E_j - E_{mid})}{RT}\right)$



The midpoint is a measure of how "easily" the species j donates or accepts e^-.

Some redox reactions

	E_{mid} (V)
$Na^+ + e^- \rightleftharpoons Na$	-2.714
$Fe^{2+} + 2e^- \rightleftharpoons Fe$	-0.440
$Fe^{3+} + 3e^- \rightleftharpoons Fe$	-0.036
$2H^+ + 2e^- \rightleftharpoons H_2$	0 (The Standard)
$AgCl + e^- \rightleftharpoons Ag + Cl^-$	+0.222
$O_2 + 4H^+ + 4e^- \rightleftharpoons H_2O$	+1.229

Intuitively, the sign of the E_{mid} gives an indication of the "natural" direction of the reactions, but this is only relative to the arbitrary standard of the hydrogen redox ($2H^+ + 2e^- \rightleftharpoons H_2$).

Finally, in the biochemical universe, redox rxns are normally coupled

E_A $A_{ox} + e^- \rightleftharpoons A_{red}$

E_B $B_{ox} + e^- \rightleftharpoons B_{red}$

$A_{ox} + B_{red} \rightleftharpoons A_{red} + B_{ox}$ (e^- transfer)

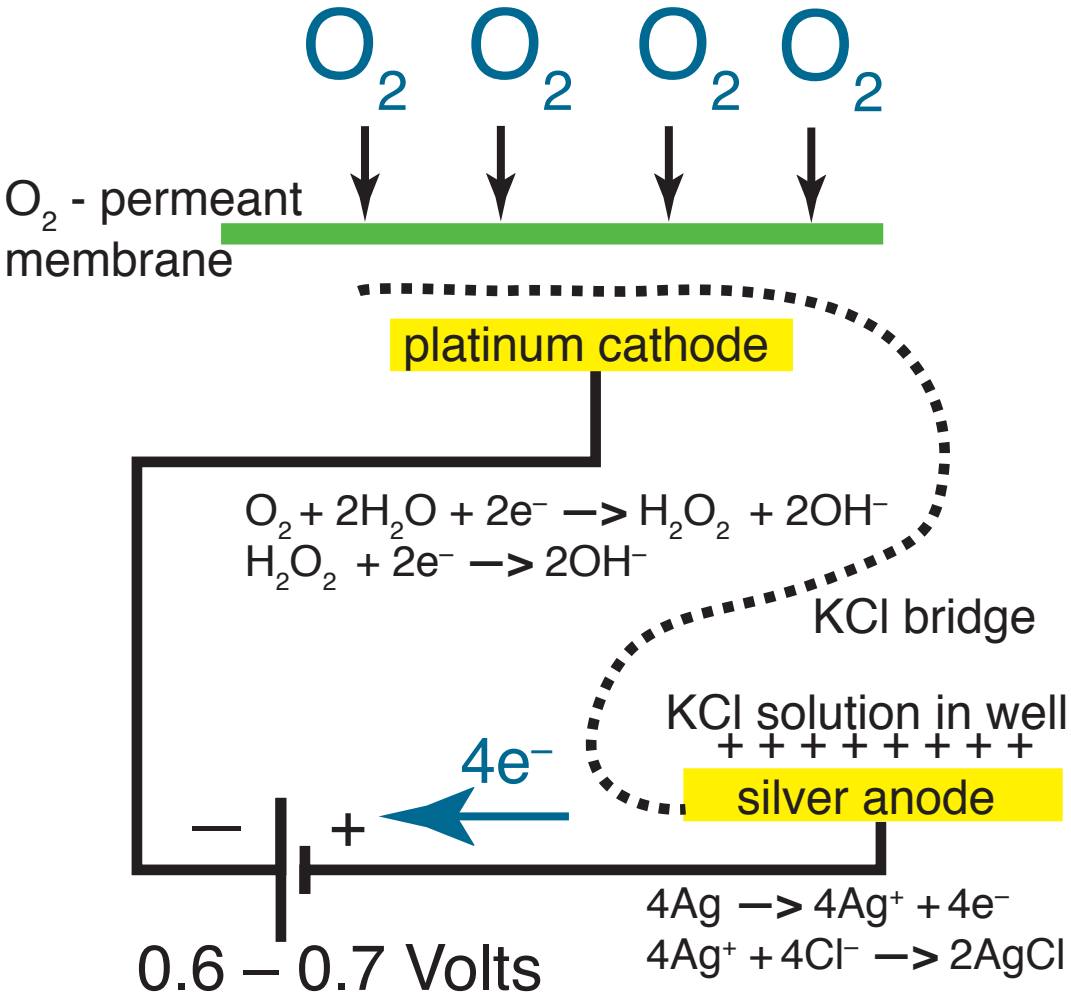
$$\Delta E = \Delta E^\circ - \frac{RT}{qF} \ln \frac{[A_{red}][B_{ox}]}{[A_{ox}][B_{red}]}$$

note that $\Delta G = -qF\Delta E$

(ΔG: Gibbs free energy)

(where ΔG is the usual description of energetics)

Redox Reactions:
The oxygen electrode (Clark-type) as a case study.



Cathode Reaction (platinum): $4e^- + O_2 + 2H_2O \leftrightarrow 4OH^-$

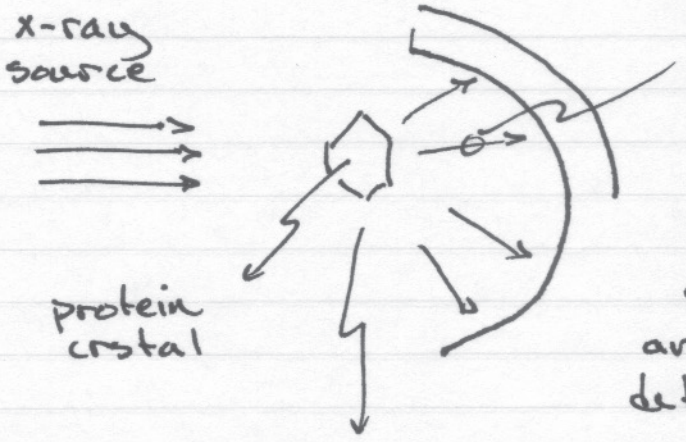
Anode Reaction (with KCl): $4Ag + 4Cl^- \leftrightarrow 4AgCl + 4e^-$

Net Result: $4e^- + O_2 + 2H_2O + 4Ag + 4Cl^- \leftrightarrow 4OH^- + 4AgCl + 4e^-$

Remember that it is not equilibrium, but 'pumped' to go to the products by the applied voltage (0.6–0.7 Volts). The e^- consumption at the Pt electrode and e^- production at the Ag electrode results in a current flow between the two half-cells —directly proportional to O_2 consumption— which is what you measure.

REACTION CENTER STRUCTURE.

The key to solving the structure is x-ray crystallography.



the x-rays are scattered when they hit atomic nuclei within the protein. This yields a distinct diffraction pattern on an x-ray sensitive detector.

The protein crstal is rotated to obtain multiple diffraction patterns. From these, computer programs are used to deduce the structure that would cause a unique set of diffraction patterns.

Nota bene: Crucial to high resolution diffraction patterns is a highly-ordered protein crystal.

This is exceedingly challenging for membrane proteins, which crystallize poorly due to their many hydrophobic residues.

Deisenhofer & Michel's accomplishments were two-fold. Successful crystallization of a membrane protein and the remarkable insights their results contributed to our understanding of photosynthesis

Thus: A Nobel Prize.

Note the remarkable array of structural alpha-helices which surround the prophyrin and other elements directly reponsible for energy transfer in the center of the reaction center.

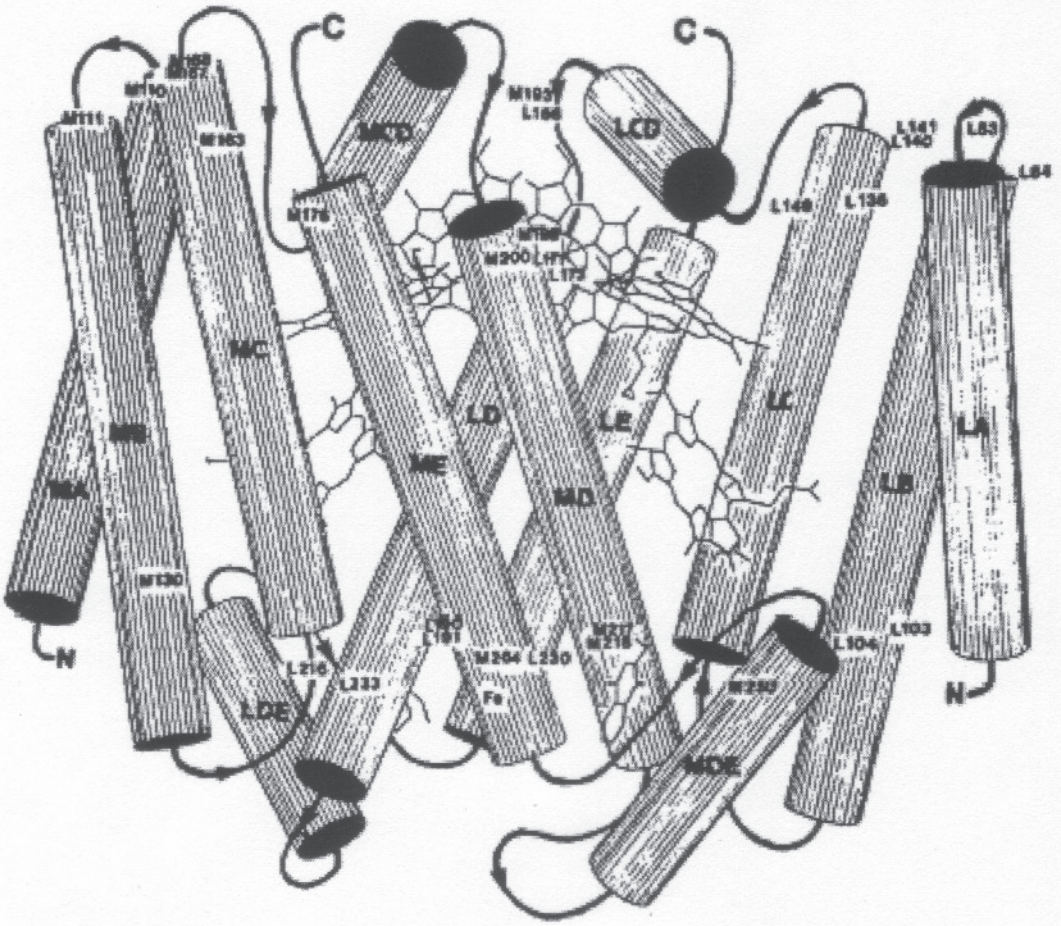


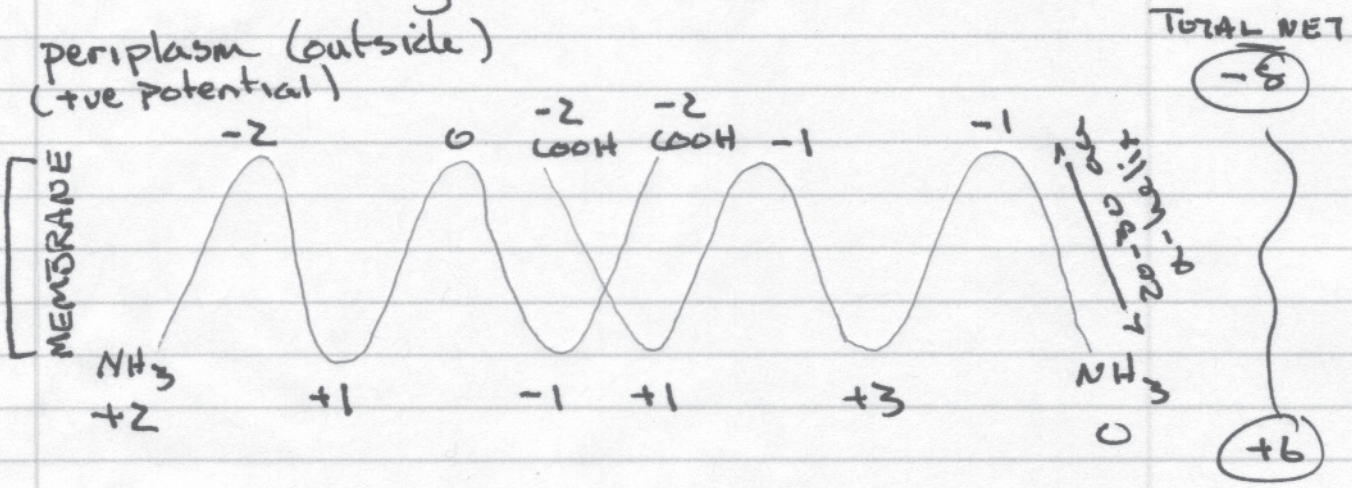
Fig. 13. Column model for the core of the reaction center from *Rps. viridis*. Only helices that are presumably conserved in photosystem II reaction centers are shown. The connections of the helices are only indicated schematically. The transmembrane helices of the L (M) subunit are labeled by LA-LE (MA-ME) and the major helices in the connections by LCD (MCD) and LDE (MDE). P's are at the interface of the L and M subunits between the D and E helices, and the BP's are near the L helices. The binding site for Q_A is between the LDE and LD helices. The location of the amino acids conserved between all L and M subunits and the D1 and D2 proteins, as well as those forming the quinone binding sites, is indicated by their sequence numbers (*42*).

Deisenhofer J. and H Michel (1989) The photosynthetic reaction center from the purple bacterium *Rhodopseudomonas viridis*. Science 245:1463-1473. [Nobel Prize Lecture].

The Rhodopseudomonas viridis reaction center revealed three major structural attributes.

One — d-helix, transmembrane segments, hydrophobic in nature dominate the structure

Two — the distribution of charged amino acids is assymetric.



THREE — charged structures were isolated in hydrophobic regions - unexpected because it is energetically unfavourable, highly so.

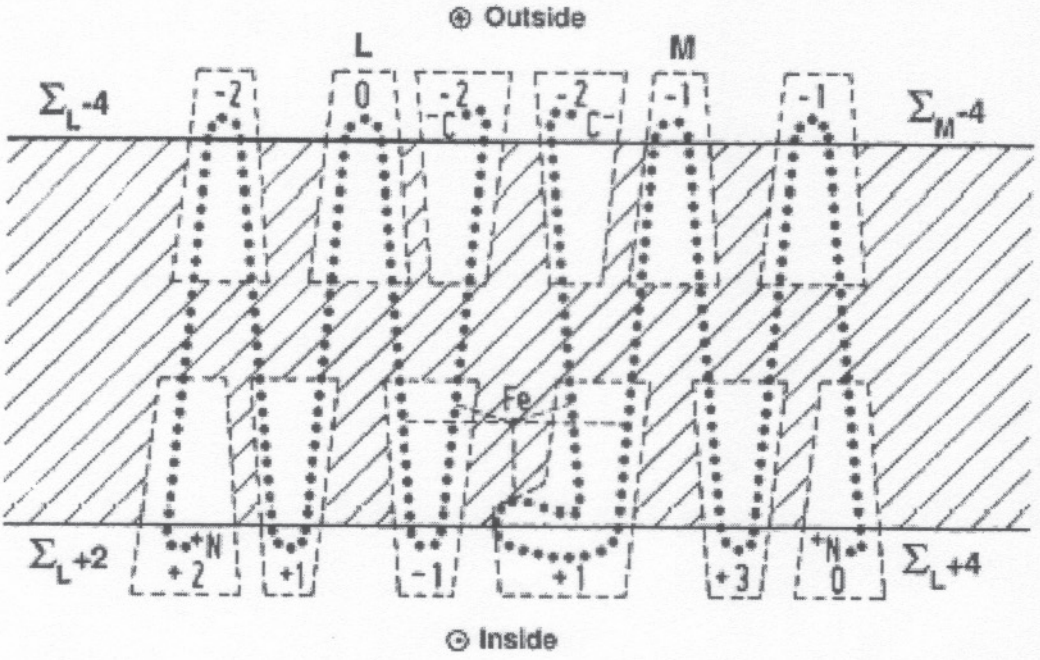


Fig. 16. Schematic drawing of the transmembrane helices, and the helix connection of the L and M subunits from *Rps. viridis* reaction center in the membrane, shows the net charges at the ends of the helices and the helix connections. The negatively charged interior of the cell is indicated by the minus sign at the bottom, the positively charged extracellular medium by the plus sign at the top (*67*).

The distribution of net electrical charges on either side of the membrane may stabilize the reaction center, since the electron transfers result in charge separation between the two sides of the membrane, a highly energetic event. This aspect of biological photosynthesis is the most difficult aspect to reproduce in bio-nanotechnology, that is, BioMimetics.

Deisenhofer J. and H Michel (1989) The photosynthetic reaction center from the purple bacterium *Rhodopseudomonas viridis*. Science 245:1463-1473. [Nobel Prize Lecture]

Photosystem II

The Rhodopseudomonas viridis reaction center is homologous (structurally as well) to the PS I reaction center of higher plants and algae.

Photosystem II structure has also been elucidated. It is a complex of a number of proteins which are expressed from either the chloroplast genome, or the nuclear genome. Most of the core proteins are chloroplast genome encoded:

PS II - A (D1)	39 kDa
PS II - B (D2)	39 kDa

create the core dimer structure, with chlorophyll, phaeophytin, quinone, carotene, and Fe.

Tyrosine and histidine side chains (+), along with Mn (manganese)

(+) $D1\text{-}tyr_{161}$ $D1\text{-}his_{190}$ & $D2\text{-}tyr_{160}$ $D2\text{-}his_{189}$

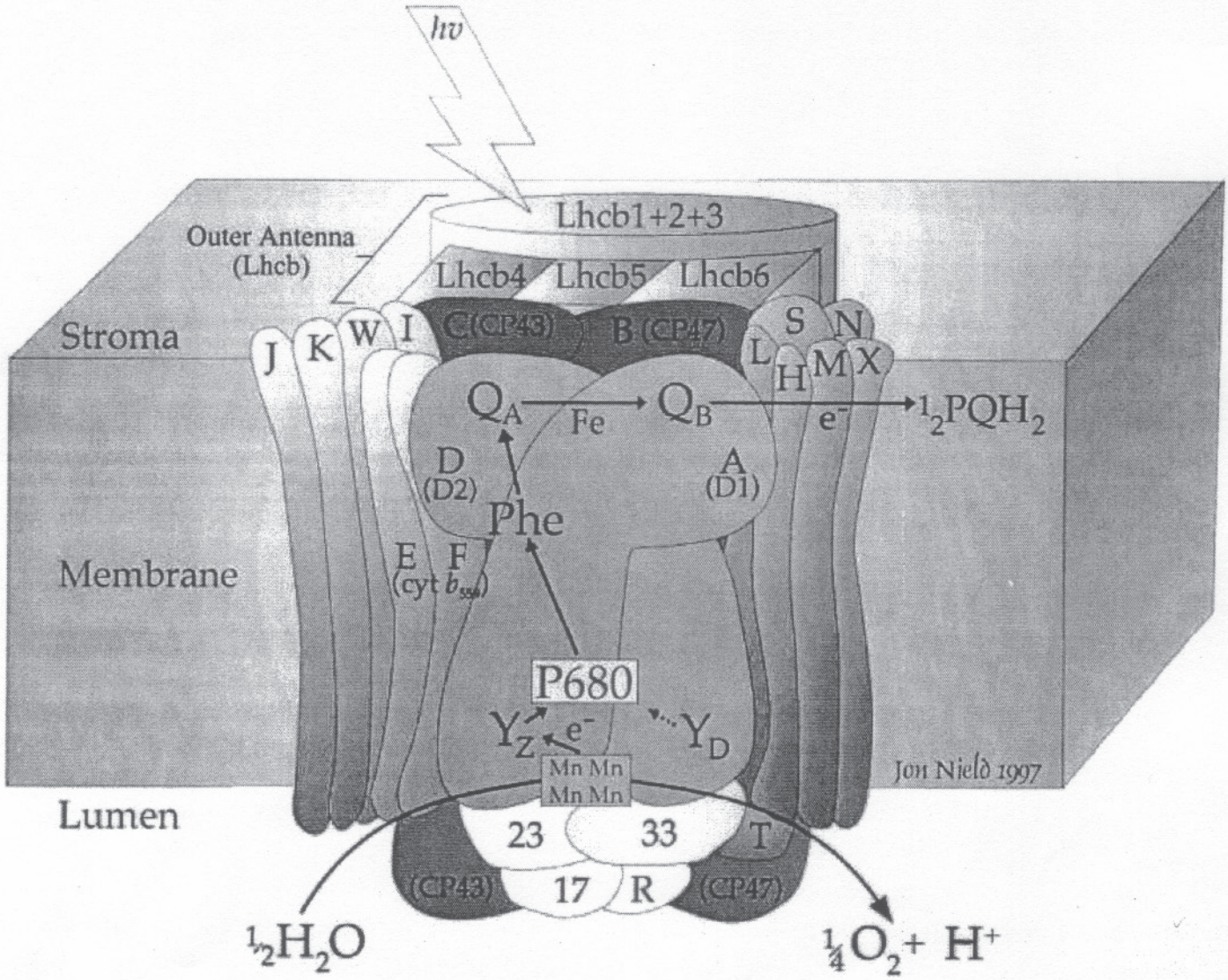
are central to a major function of PS II, the extraction of e^- from H_2O, producing H^+ and O_2

H^+ → ATP synthesis from pmf

O_2 → waste product.

Protein subunits of PSII - Barber et al. (1997) Physiol. Plant. 100:817-827

At present there are 25 genes which have been identified as encoding proteins for the PSII core and are referred to as psb (photosystem b) genes. In higher plants and algae, most of these genes are located in the chloroplast genome, but some are nuclear encoded. There are undoubtedly more to be discovered. In some cases these components are restricted to a particular class of organism. In addition there are the genes that encode the proteins of the outer antenna systems; cab genes in higher plants and green algae give rise to a series of chlorophyll a/chlorophyll b binding proteins (Lhcb1-6) while the apc and cpc genes encode the protein of the phycobilisomes of cyanobacteria and red algae.



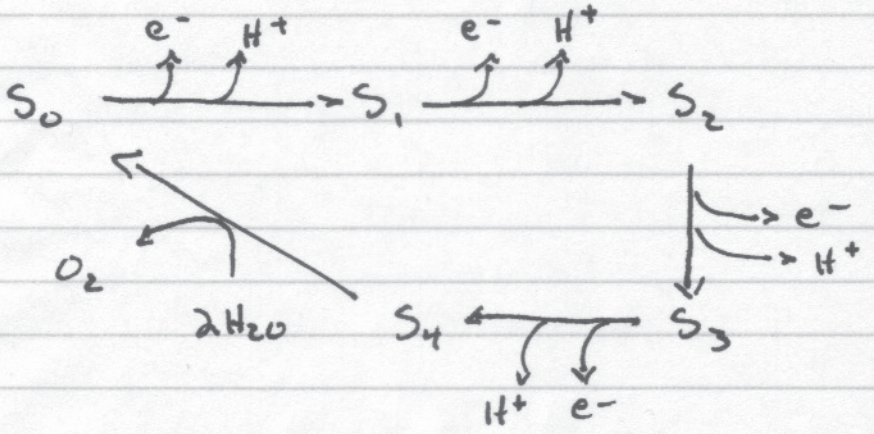
from:http://www.bio.ic.ac.uk/research/barber/photosystemII/PSII-subunits.html The Barber Research Group at Imperial College of Science, Technology and Medicine. London UK.

WATER SPLITTING.

The general mechanism involves stepwise oxidation of the complex, such that, after 4 "photonic events":

$$2H_2O + S^{4+} \longrightarrow S + 4H^+ + O_2. \quad \text{(overall)}$$

in step-wise fashion:



The S-mechanism was originally envisaged based upon flashed light experiments, in which maximal O_2 production was observed every third flash.

Mn, Ca, and Cl are co-factors. But still, the mechanism is not completely elucidated, and may not be until it is built from scratch in vitro

The most recent advances, which have relied upon time resolved x-ray absorption spectroscopy using x-ray beams at a third generation synchrotron light source in Grenoble France.

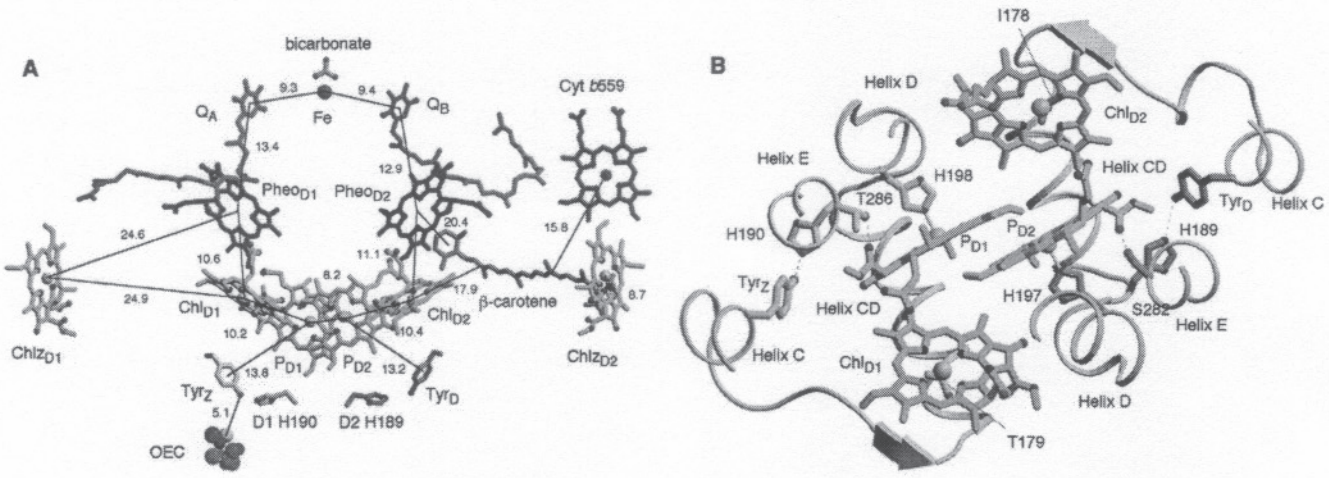


Fig. 2. Cofactors involved in electron transfer. (A) Electron transfer cofactors shown perpendicular to the internal pseudo-twofold. The phytol tails of the chlorophylls and pheophytins have been removed for clarity. The side chains of TyrZ (D1 Tyr161) and D1 His190 are shown, and TyrD (D2 Tyr160) and D2 His189. The four chlorophylls comprising P680 are in direct van der Waals contact, and other electron transfer distances are given in Å. (B) The P680 dimer of chlorophylls (PD1 and PD2) and accessory Chls (ChlD1 and ChlD2). The histidine ligands D1 His198 and D2 His197 are shown, as well as the redox-active TyrZ–D1 His190 and TyrD–D2 His189 pairs. The view is down the pseudo-twofold axis from the stromal side.

Source: Science 19 March 2004: Vol. 303. no. 5665, pp. 1831 - 1838
DOI: 10.1126/science.1093087

Architecture of the Photosynthetic Oxygen-Evolving Center

Kristina N. Ferreira, Tina M. Iverson, Karim Maghlaoui, James Barber, and So Iwata

Water splitting and cycle of S states[1]

When dark-adapted algae are illuminated with short flashes of bright light, separated by darkness, a characteristic pattern of O_2 evolution results. The first two flashes evolve little or no O_2, the third a large 'gush' and the fourth a smaller amount of O_2 than the third but more than the first, that is, a periodicity of four.

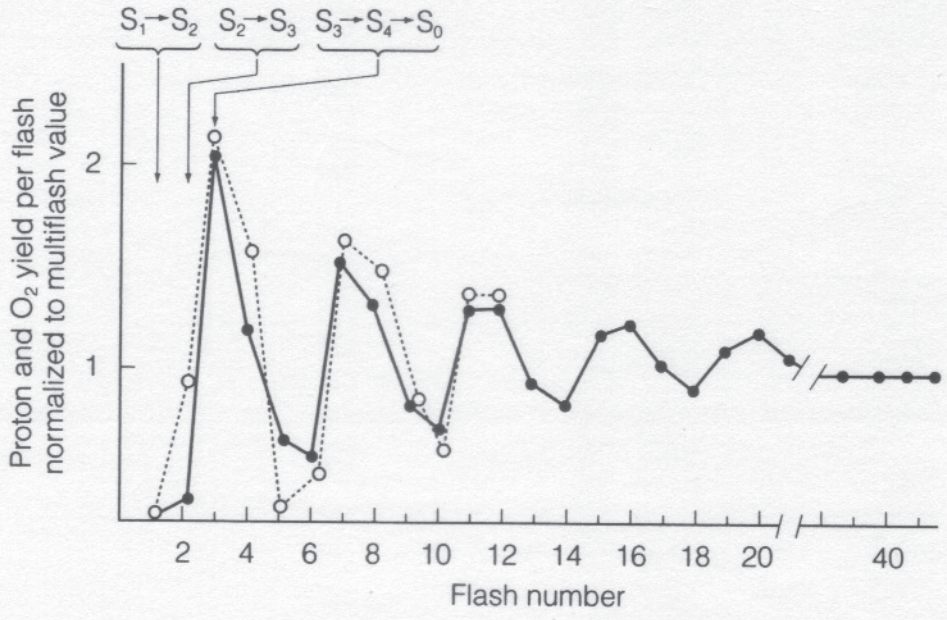
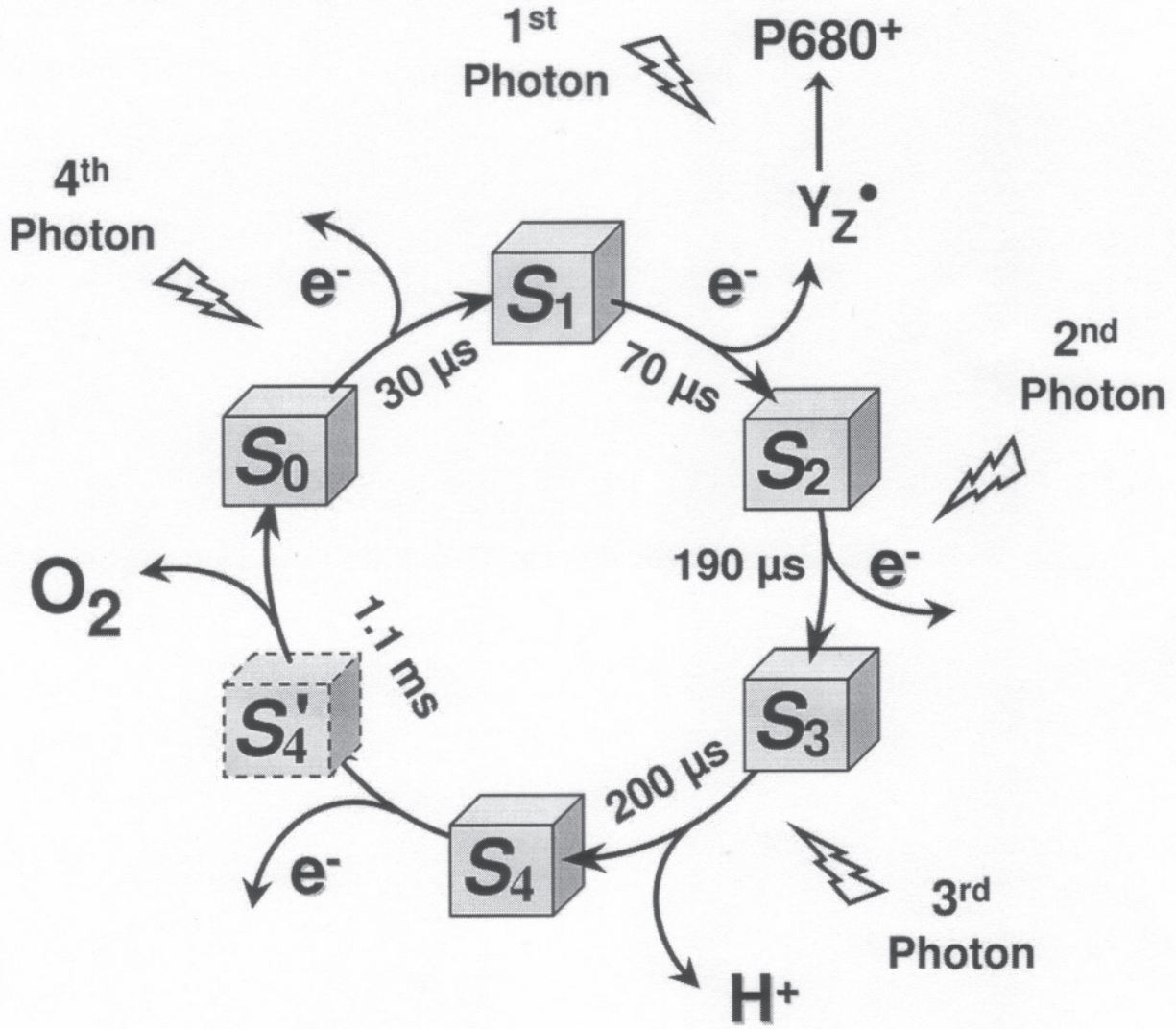


Figure 5.6. Oxygen evolution and proton release by chloroplasts given short (2 μs) intense flashes of light separated by darkness. The number per flash is expressed relative to the production after many flashes. (●—●) O_2 evolution; (○– –○) H^+ evolution

[1] Source: DW Lawlor (2001) Photosynthesis. 3[d] edition. Springer-Verlag. pp. 38–39.

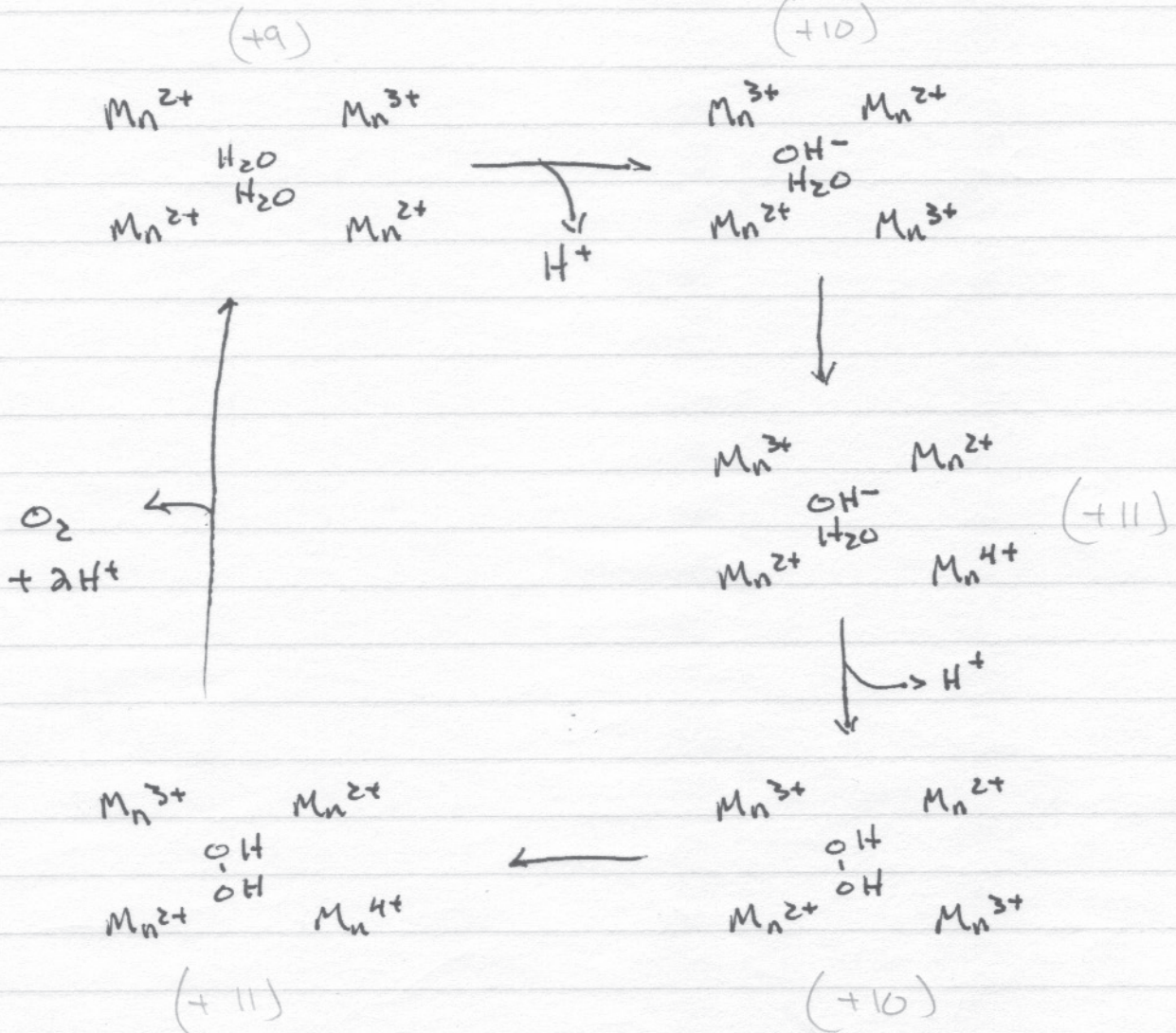
Extension of the classic S-state cycle of photosynthetic oxygen evolution[1]

The classic S-cycle model has been proposed by Kok on the basis of the flash-number dependence of the O_2 yield that was first observed by Joliot and Joliot. The oxygen-evolving complex (OEC) at the PSII donor side comprises a manganese-calcium complex (4 Mn and 1 Ca) and its protein environment. Often also a nearby tyrosine (Y_Z•) is included as an integral part of the OEC (1–6). Driven by the sequential absorption of four light quanta, which in the present study were provided by four laser flashes, the OEC is stepped through its reaction cycle. After absorption of a photon, a chlorophyll cation ($P680^+$) is formed, which oxidizes Y_Z. The tyrosine radical (Y_Z•) then extracts one electron from the Mn complex. The S_1 state is dark-stable; S_2 and S_3 are formed by one and two light-driven oxidation steps, respectively. The third photon induces the S_3—>S_0 transition and dioxygen is released; the fourth photon closes the cycle. Proton release not representing a distinct, rate-limiting step has been omitted. Existence and formation rate of the S_4 state are uncovered in the present investigation. The S_4 intermediate is not formed by electron transfer to Y_Z• but by a deprotonation reaction. In S_4, four oxidizing equivalents have been accumulated by the OEC, including Y_Z•. The classic S-state cycle is extended by the S_4' state that represents a hypothetical intermediate in which four electrons have been extracted from the Mn complex, including Mn ligands and the two substrate water molecules.



[1] Source: M Haumann, P Liebisch, C Müller, M Barra, M Grabolle H Dau 2005 Photosynthetic O_2 formation tracked by time-resolved X-ray experiments. Science 310:1019–1021.

WATER SPLITTING



A possible scheme, focussed on the Mn complex.

Volkov & Brown 2006 Electrochemistry of Plant life. *in* Volkov (ed.) Plant Electrophysiology Springer-Verlag pp. 455.

WATER SPLITTING

X-ray fluorescence can be used to resolve oxidation and reduction of the Mn complex of PS II

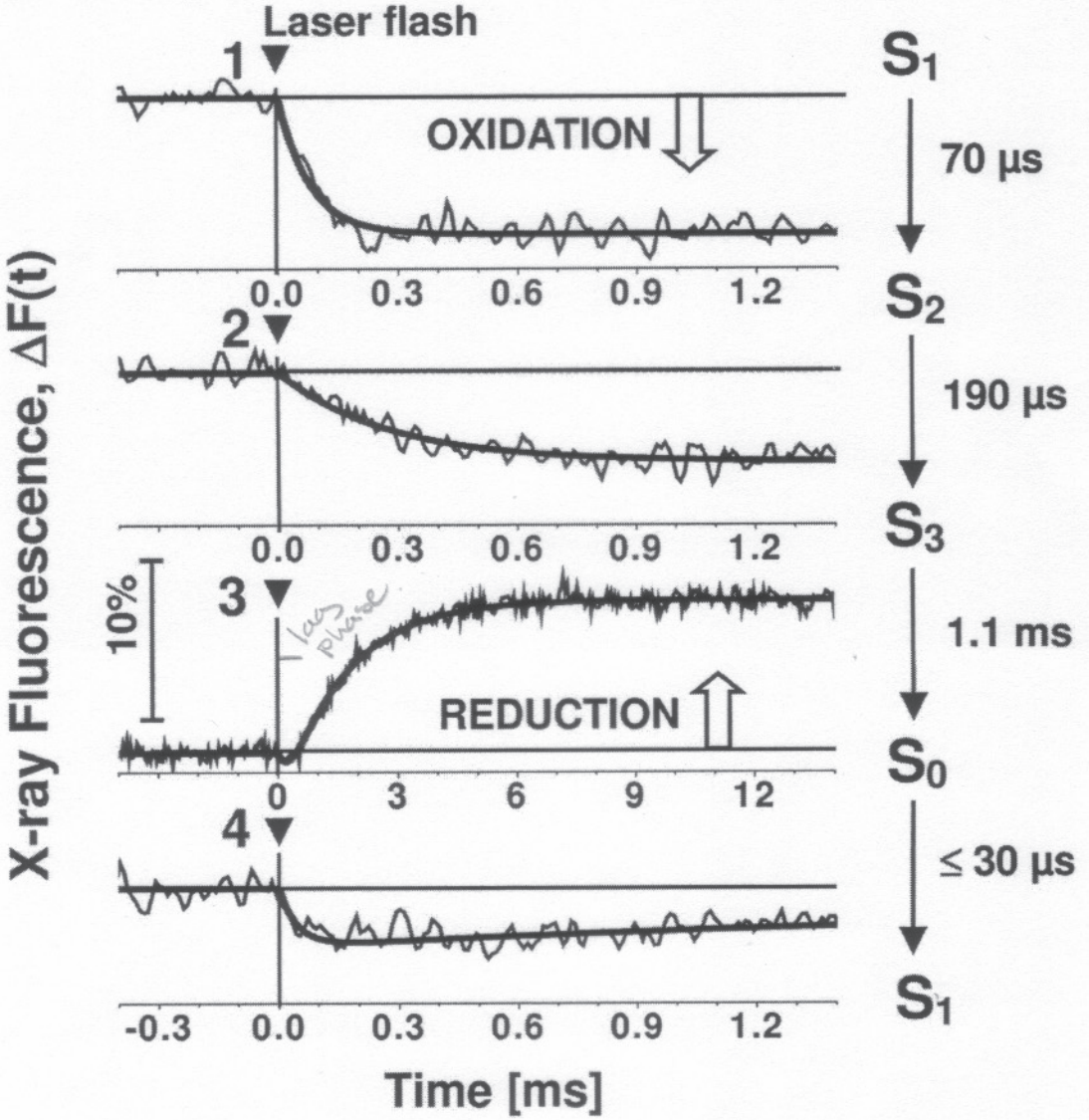
In these experiments, a light flash technique was used to separate the different S-states, but what was monitored was the redox state of Mn.

In addition to establishing a redox-state cycle consistent with the cycle of O_2 evolution, Haumann et al. identified a transient S_4' state just prior to O_2 release.

It still remains unclear what the mechanism of water-splitting is in toto, but step-by-step, it is being illuminated (pun intended).

Oxidation and reduction of the Mn complex of PSII monitored by time-resolved x-ray measurements.[1]

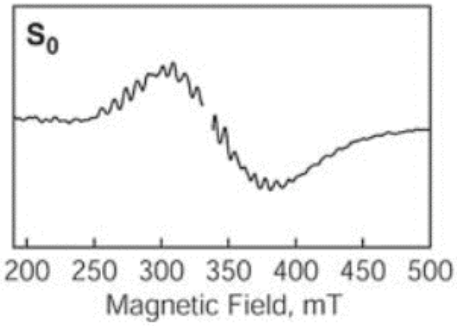
Facilitated by the high flux and stability of the x-ray beam at a third-generation synchrotron (European Synchrotron Radiation Facility, beamline ID26, Grenoble, France), we could follow changes in the Mn x-ray fluorescence after laser-flash illumination of PSII with a time resolution of 10 μs. For S_1->S_2 (first flash) and S_2->S_3 (second flash) the exponential absorption decrease indicated oxidation of the Mn complex by the tyrosine radical Y_Z• with halftimes of 70 μs and 190 μs, respectively; for S_0->S_1 (fourth flash), the $t_{1/2}$ was ≤30 μs. For S_3—>S_0 (third flash), the laser flash induced an absorption increase due to Mn reduction by the substrate water ($t_{1/2}$ = 1.1 ms); however, this was preceded by a lag phase of about 250 μs (Fig. 2). This lag phase suggested a kinetically resolvable intermediate. However, for transients collected at 6552 eV, it could not be unambiguously assigned to an intermediate in the S_3—>S_0 transition. …The intermediate is formed before the Mn-reducing/O_2-forming step and thus represents the long-searched-for S_4 state.



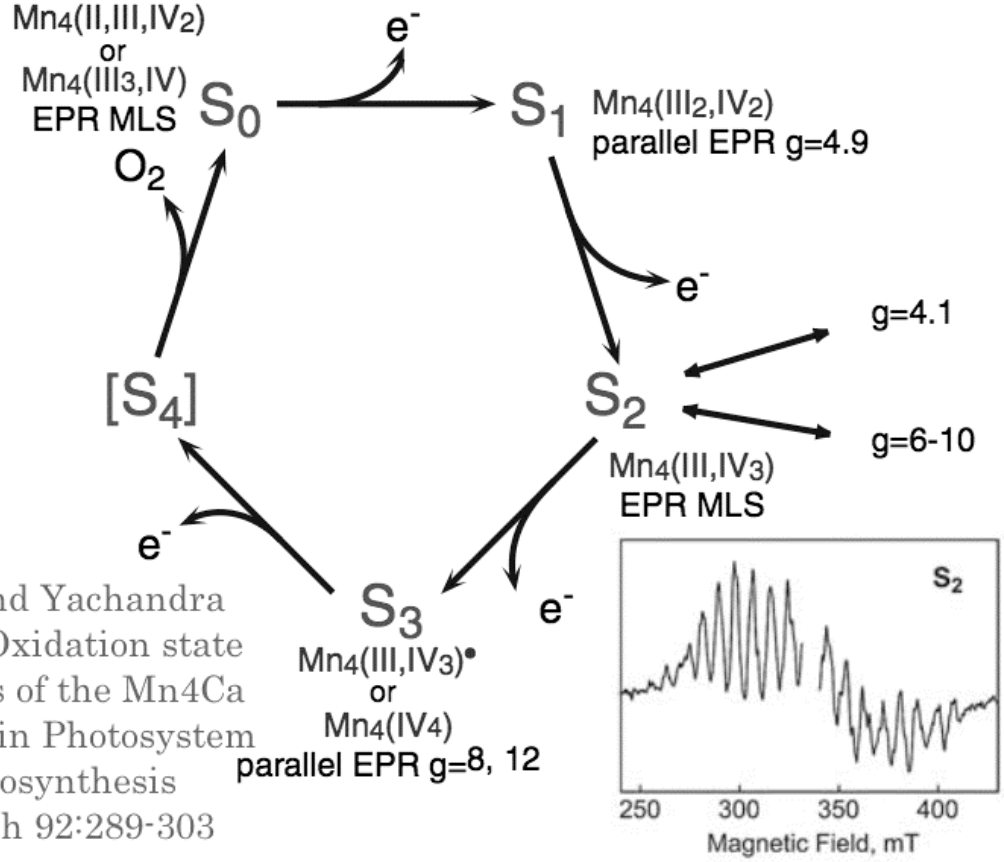
oxidation of the Mn complex by tyrosine radical $Y_Z^{\bullet}$

Mn couple reduction by water

[1] Source: M Haumann, P Liebisch, C Müller, M Barra, M Grabolle H Dau 2005 Photosynthetic O_2 formation tracked by time-resolved X-ray experiments. Science 310:1019–1021.



The S-state cycle and the proposed oxidation states of the Mn-cluster in the S-states. The multiline EPR signals for the S0 and S2 states and the spin states identified with S0, S1, S2, and S3 (for spinach) are also shown.

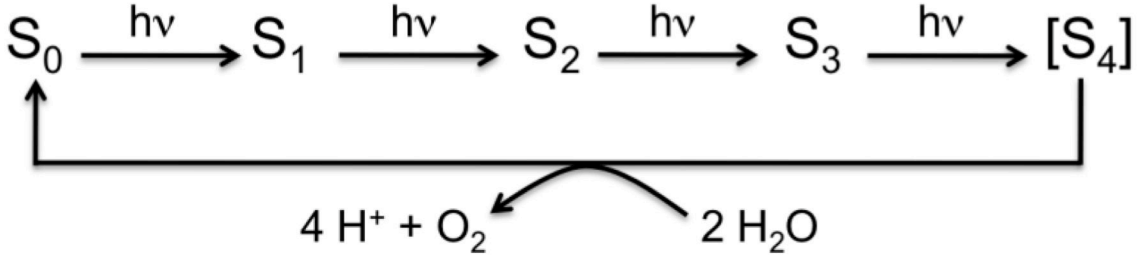


Yano and Yachandra (2007) Oxidation state changes of the Mn4Ca cluster in Photosystem II. Photosynthesis Research 92:289-303

Simultaneous Femtosecond X-ray Spectroscopy and Diffraction of Photosystem II at Room Temperature

Jan Kern,[1,2] Roberto Alonso-Mori,[2] Rosalie Tran,[1] Johan Hattne,[1] Richard J. Gildea,[1] Nathaniel Echols,[1] Carina Glöckner,[3] Julia Hellmich,[3] Hartawan Laksmono,[4] Raymond G. Sierra,[4] Benedikt Lassalle-Kaiser,[1]* Sergey Koroidov,[5] Alyssa Lampe,[1] Guangye Han,[1] Sheraz Gul,[1] Dörte DiFiore,[3] Despina Milathianaki,[2] Alan R. Fry,[2] Alan Miahnahri,[2] Donald W. Schafer,[2] Marc Messerschmidt,[2] M. Marvin Seibert,[2] Jason E. Koglin,[2] Dimosthenis Sokaras,[6] Tsu-Chien Weng,[6] Jonas Sellberg,[6,7] Matthew J. Latimer,[6] Ralf W. Grosse-Kunstleve,[1] Petrus H. Zwart,[1] William E. White,[2] Pieter Glatzel,[8] Paul D. Adams,[1] Michael J. Bogan,[2,4] Garth J. Williams,[2] Sébastien Boutet,[2] Johannes Messinger,[5] Athina Zouni,[3] Nicholas K. Sauter,[1] Vittal K. Yachandra,[1]† Uwe Bergmann,[2]† Junko Yano[1]†

One Protein, Two Probes. A central challenge in the use of x-ray diffraction to characterize macromolecular structure is the propensity of the high-energy radiation to damage the sample during data collection. Recently, a powerful accelerator-based, ultrafast x-ray laser source has been used to determine the geometric structures of small protein crystals too fragile for conventional diffraction techniques. **Kern *et al.*** (Science 340:491–495 [2013]) now pair this method with concurrent x-ray emission spectroscopy to probe electronic structure, as well as geometry, and were able to characterize the metal oxidation states in the oxygen-evolving complex within photosystem II crystals, while simultaneously verifying the surrounding protein structure.



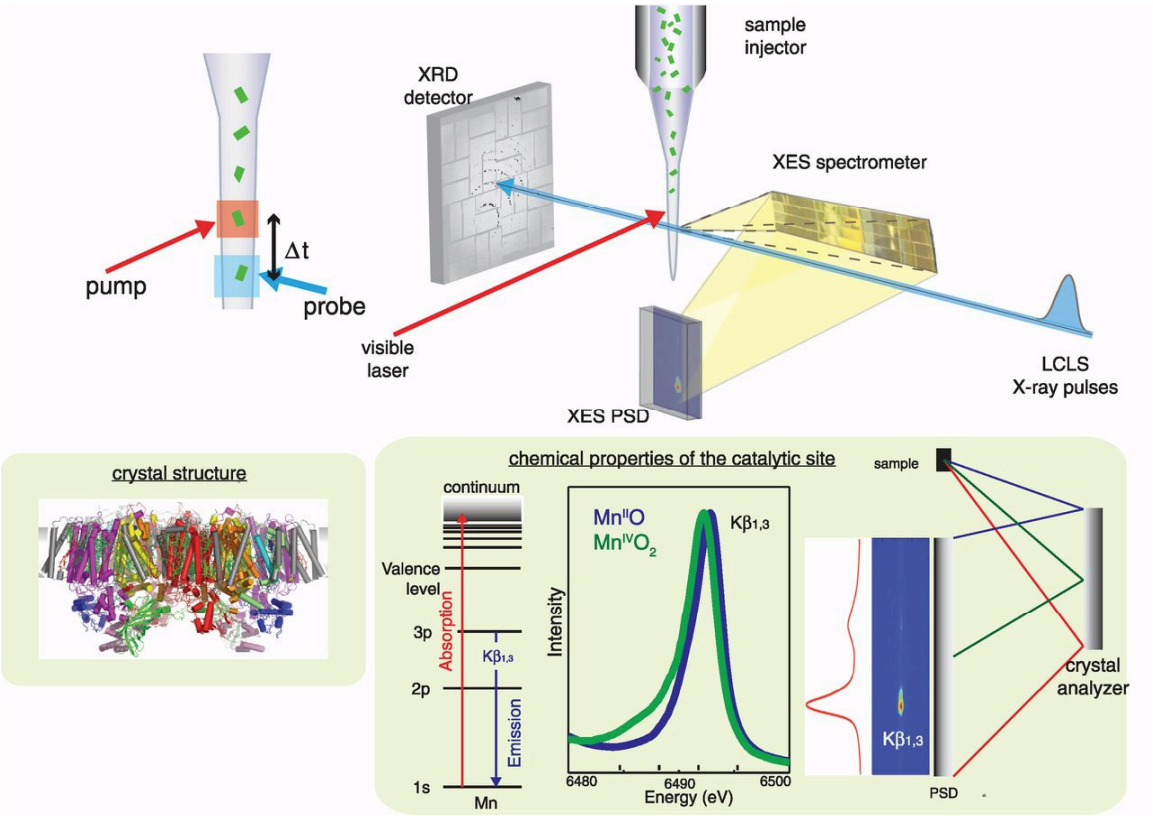


Fig. 1 Setup of simultaneous x-ray spectroscopy and crystallography experiment using femtosecond x-ray pulses.

The crystal suspension is electric-field–focused into a microjet that intersects the x-ray pulses. X-ray diffraction data are collected at the XRD detector. X-ray fluorescence is collected from the same crystal are collected at ~90° by the XES spectrometer and a compact position-sensitive detector (PSD). A 527 nm laser is used to illuminate the crystals. The timing protocol (top left) consists of a fixed time of flight Δt between the optical pump and x-ray probe. The schematic of the energy dispersive spectrometer is shown (bottom right), as well as the Mn^{II} and Mn^{IV} oxide $K\beta_{1,3}$ spectra and an energy-level diagram for XES (bottom middle).

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The technique allows both structure and the oxidation state of the manganese atoms to be collected simultaneously.

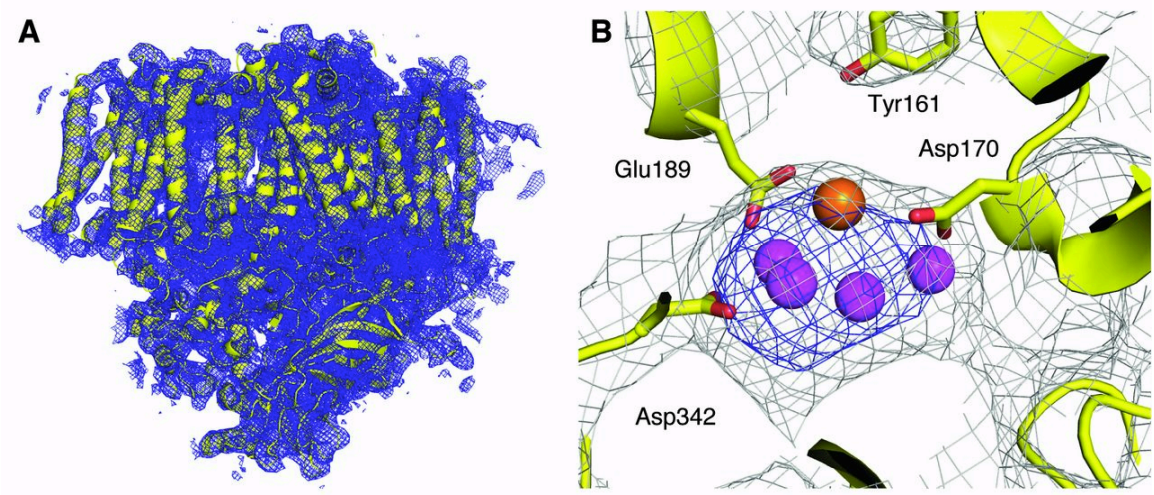

Fig. 2 Structure deduced from diffraction of micrometer-sized crystals of PS II using sub–50-fs x-ray pulses at room temperature.

(**A**) $2mF_o$-DF_c electron density map for the PS II complex in the dark S_1 state. One monomer of the protein is shown in yellow, and the electron density is contoured in blue mesh. (**B**) Detail of the same map in the area of the Mn_4CaO_5 cluster in the dark S_1 state. Selected residues from subunit D1 are labeled for orientation; Mn is shown as purple spheres and Ca as an orange sphere.

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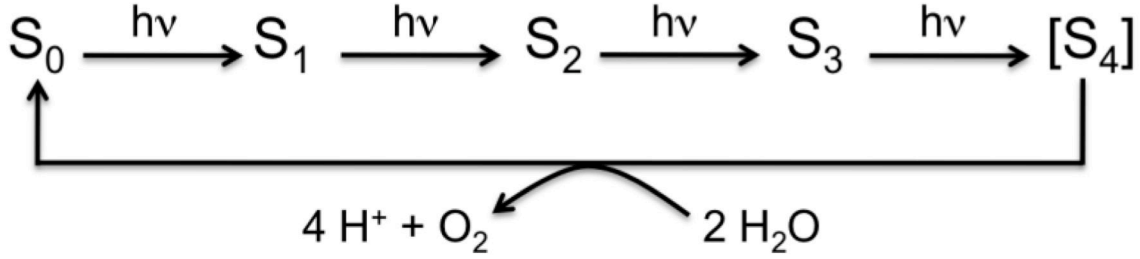
The resolution is not great (because it's being done at room temperature), but it is a major breakthrough.



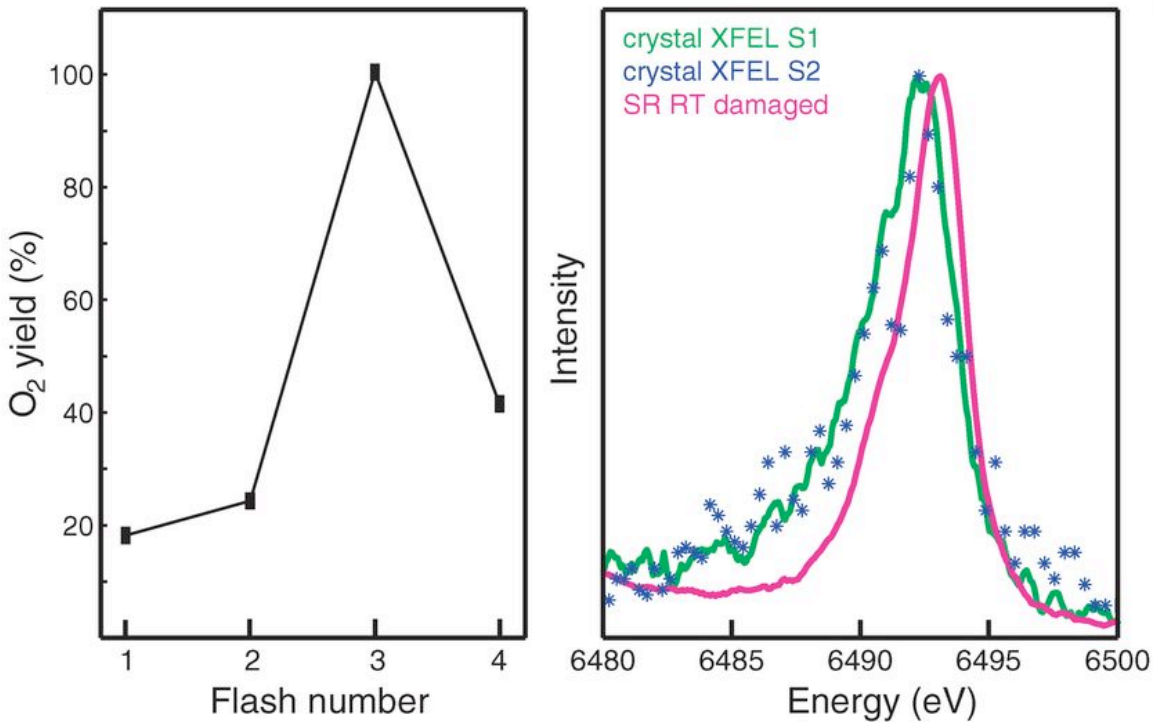


Fig. 4 Characterization of the illuminated PS II sample.

(**A**) Light-induced O_2 yield detected as mixed labeled $^{16}O^{18}O$ species after illumination of photosystem II. More than 73% of the sample occupies the S_2 state after one illumination. (**B**) X-ray fluorescence of PS II in the S_2 state. PS II in the first illuminated S_2 state is shown in blue (asterisks). PS II in the dark stable S_1 state is shown in green. Completely photoreduced ("damaged") PS II collected at room temperature is shown in pink.

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The results so far validate the technique, using the water-splitting enzyme as the model test system. The unraveling of the step-by-step splitting of water remains to be explored.